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Formation of atypical fruiting structures in *Ganoderma lucidum* isolates on a nutrient agar medium

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Effects of light and ventilation on the formation of atypical fruiting structures (AFSs) and fruit body primordia (FBPs) of *Ganoderma lucidum* on nutrient agar media were investigated. Although the mycelial growth was inhibited by illumination and ventilation, brown AFSs appeared on the white mycelial colony, and basidia and basidiospores were produced on the AFSs. On the other hand, FBPs were induced by illumination alone, regardless of ventilation. However, the primordia could not develop to mature fruit bodies. In the dark, only vegetative growth of the fungus progressed. Twenty-three isolates of *G. lucidum* collected from four countries were tested for the formation of AFSs and FBPs under light and ventilation. Thirteen isolates formed AFSs, and another five isolates produced FBPs. Of the remaining five isolates, one formed callus-like structures without elaborating basidiospores, and the other four did not induce AFSs or FBPs. Microscopical observation showed that the basidia were formed directly from generative hyphae on the surface of AFSs. Basidiospores formed on the basidia were brown and ellipsoid with an eccentric hilar appendix on the rounded spore base. They had a double wall and mostly contained one or two large vacuoles. The surface of basidiospores was smooth or wrinkled and had shallow holes. The spore size was (4.5-)6.4-9.6(-10.3) × (2.6-)3.2-5.1(-6.4), 7.3 × 4.2 μm on average.

Key Words——atypical fruiting structure; basidiospore; fruit body primordium; *Ganoderma lucidum*; light.

Introduction

Ganoderma lucidum (Fr.) Karst., a stalked mushroom with porous hymenium, which belongs to the white-rot basidiomycetes, has long been used as a traditional medicine in China, Japan and Korea. Artificial cultivation of *G. lucidum* using bottles with sawdust and timber logs was established in Japan (Hemmi and Tanaka, 1936; Nanamiya, 1982). In Korea, the artificial cultivation for farm production was started in 1985 (Seo, 1987), and the cultivated area has been increasing.

In artificial culture, *Ganoderma* spp. show various characteristics of the hyphae, such as generative hyphae with clamp-connections, fiber or skeletal hyphae, staghorn hyphae, cuticular cells and vesicles, and hyphal rosettes in *G. zonatum* Murrill (Seo, 1987; Adaskaveg and Gilbertson, 1989). In addition, Shin and Seo (1988a) found that *G. lucidum* produces atypical fruiting structures (AFSs) without the formation of basidiocarps on several nutrient agar media under light illumination. Basidia developed from the surface of AFSs, and basidiospores were produced on the basidia. On the other hand, this fungus produces fruit body primordia (FBPs) on nutrient agar media under light (Shin and Seo, 1988a; Adaskaveg and Gilbertson, 1989). However, it is still unclear whether the formation of AFSs and FBPs on agar media is a common feature of *G. lucidum* strains.

This paper discribes the formation of AFSs and FBPs in *G. lucidum* isolates collected from four countries, and the morphology of AFSs and non-basidiocarpous basidiospores.

Materials and Methods

Isolates The dikaryon isolates of *G. lucidum* used in this study are listed in Table 3. Eleven isolates were obtained from the context tissue of wild-type fruit bodies in Korea (nine isolates) and Papua New Guinea (two isolates). Two isolates were obtained from artificial log-cultivation of the mushroom in Korea. Other isolates were presented by the Agriculture Science Institute, Korea (five isolates; two isolates obtained from wild-type fruit body in Korea and three isolates from Japan), Tottori Mycological Institute, Japan (two isolates) and the Mushroom Research Institute, University of Pennsylvania, U.S.A. (three isolates).

Culture condition All isolates were cultured and maintained on a nutritionally complete agar medium (CM) composed of 0.5 g $MgSO_4 \cdot 7H_2O$, 0.46 g KH_2PO_4, 1 g K_2HPO_4, 2 g peptone, 2 g yeast extract, 20 g glucose, and 20 g agar per liter of distilled water (Raudaskoski and Viitanen, 1982). Mycelial disks (6 mm in diameter) from cultures were placed in 90-mm plastic petri dishes containing about 30 ml of CM, and were incubated at 27 ± 1 °C for 30 days under continuous illumination with daylight fluorescent lamps (FL 20 SD, Matsushita) or in

Table 1. Effect of light and ventilation on mycelial growth of *Ganoderma lucidum*.

Treatment*	GI-009		GI-010	
	Mycelial growth** (mm)	Inhibition rate*** (%)	Mycelial growth (mm)	Inhibition rate (%)
Dark Sealed	77.8±0.4		69.3±5.6	
Unsealed	63.2±2.4	18.8± 3.1	33.2±2.4	52.2±3.5
Light Sealed	54.2±9.3	30.4±11.9	23.2±3.4	66.6±4.8
Unsealed	25.0±1.3	69.9± 1.6	18.4±1.4	73.5±2.0

* Isolates (GI-009 and GI-010) were incubated for 7 days under daylight fluorescent lamp (5.0 μmol $m^{-2}s^{-1}$) or in the dark on CM in sealed or unsealed petri dishes.

** All cultures were replicated five times, and each value shows the mean with standard deviation.

*** Inhibition rate (%) $= \left(1 - \frac{\text{Mycelial growth under dark-unsealed, light-sealed or light-unsealed conditions}}{\text{Mycelial growth under dark-sealed condition}}\right) \times 100$

the dark. The light intensity was adjusted to about 5.0 μmol $m^{-2}s^{-1}$ by controlling the distance between the lamps and the petri dishes. Light intensity was determined with a LI-189 Quantum-meter (LI-COR, Inc). To examine the effect of ventilation on formation of AFS and FBP, the lids of petri dishes were doubly sealed with parafilm (American National Can$_{TM}$) after inoculation. Dishes were incubated for 30 days in light or dark conditions.

Microscopical observation The formation of AFSs and non-basidiocarpous basidiospores in *G. lucidum* was monitored under a stereo- and light microscope (Optiphoto, Nikon). Cultures were also observed under a scanning electron microscope (SEM) (X-650, Hitachi). For SEM observation, about 5-mm square plugs of AFSs were removed from cultures with a razor blade. Samples were fixed using 2.5% glutaraldehyde and post-fixed in 2% osmium tetroxide (Adaskaveg and Gilbertson, 1986; Mims and Seabury, 1989). They were then dehydrated in a series of 30 to 100% ethanol, and replaced the ethanol with a series of 50, 75, and 100% amylacetate. Finally, plugs were dried in carbon dioxide using a critical-point dryer (HCP-2, Hitachi), mounted on specimen stubs with adhesive silver paste, and coated with gold to a thickness of about 15 nm using a sputter multicoater (VX-10A, Eiko).

Results

Effect of light and ventilation on formation of AFS and FBP To determine the conditions for formation of AFSs and FBPs, isolates GI-010 and GI-009, which form AFSs and FBPs, respectively, were selected from a preliminary experiment. These isolates were grown on CM for 30 days with or without ventilation in light or dark conditions. Mycelial growth of both isolates was inhibited by light and/or ventilation (Table 1). Mycelial mats became dense with ventilation, and flattened with yellowish patches in light. With light and ventilation, brown AFSs appeared on the white mycelial colony of isolate GI-010 after about 10 days, and developed with incubation time (Table 2; Figs. 1, 2). AFSs were of two types, coralloid and incomplete poroid (Figs. 1–4), of which both types appeared in each experiment repeated several times. Basidia and basidiospores were formed on the surface of both types of AFS (Figs. 5, 6). In isolate GI-009, FBPs were induced by light in about 10 days, regardless of ventilation (Table 2; Fig. 7). However, primordia could not develop to mature fruit bodies with basidiospores. In the dark, both isolates continued only vegetative growth even after 30 days (Table 2; Fig. 8).

Formation of AFS and FBP in *G. lucidum* isolates Twenty-three isolates of *G. lucidum* collected from four countries were tested for formation of AFSs and FBPs on CM under light and ventilation (Table 3). Of these, nine isolates from Korea, three from Japan and one from Papua New Guinea formed AFSs with basidia and basidiospores. The formation of FBPs was observed in two isolates from Korea, two isolates from Japan and one isolate from the U.S.A. Isolates forming both AFSs and FBPs were not detected. In one isolate from Korea, a callus-like structure differing in form from AFS and FBP appeared on the colony, but formation of basidia and basidiospores was not induced. Two isolates from the U.S.A., one isolate from Korea and an isolate from Papua New Guinea showed only vegetative growth. No isolates formed AFSs or FBPs in the dark.

Morphology of AFSs and basidiospores The AFSs of GI-010 isolate incubated for 30 days under light and ventilation were observed microscopically. The isolate had

Figs. 1–8. Cultures of *G. lucidum* incubated on a complete medium for 4 weeks in light under ventilation. Scales = 3 mm for Figs. 3 and 4; 5 μm for Figs. 5 and 6. 1. Coralloid-type AFSs formed on mycelial colony of isolate GI-010. 2. Poroid-type AFSs formed on mycelial colony of isolate GI-010. 3. Magnification of coralloid-type AFS on arrowhead of Fig. 1. 4. Magnification of poroid-type AFS on arrowhead of Fig. 2. 5. Basidium and basidiospores formed on AFS of isolate GI-010 observed by light microscopy. Basidiospores have one or two vacuoles, and the basidium also has large vacuoles. 6. Basidiospores of isolate GI-010 observed by light microscopy. Basidiospores have double walls and large vacuoles. 7. FBPs formed on mycelial colony of isolate GI-009. 8. Culture of isolate GI-010 incubated for 4 weeks in the dark. The isolate grew only vegetatively.


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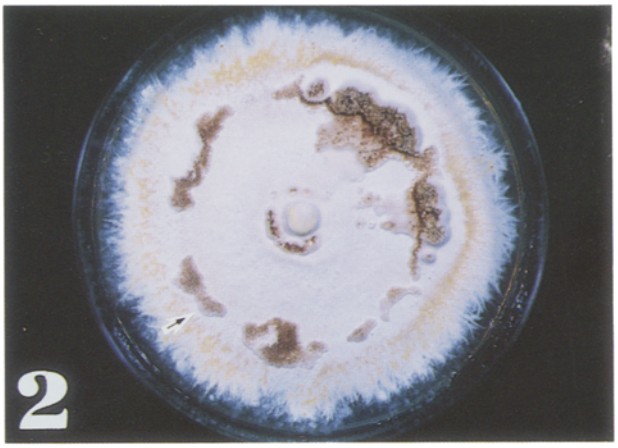
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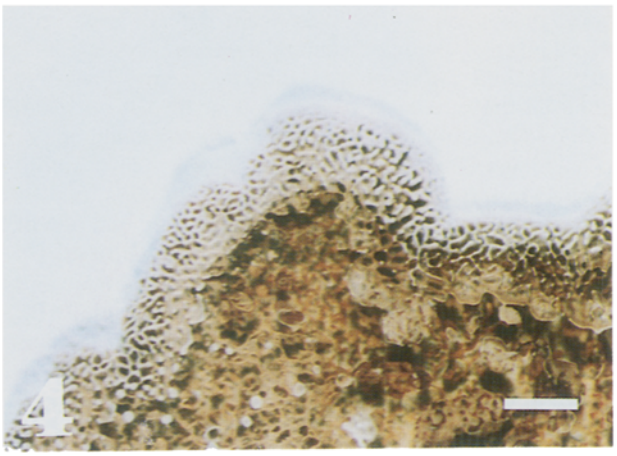
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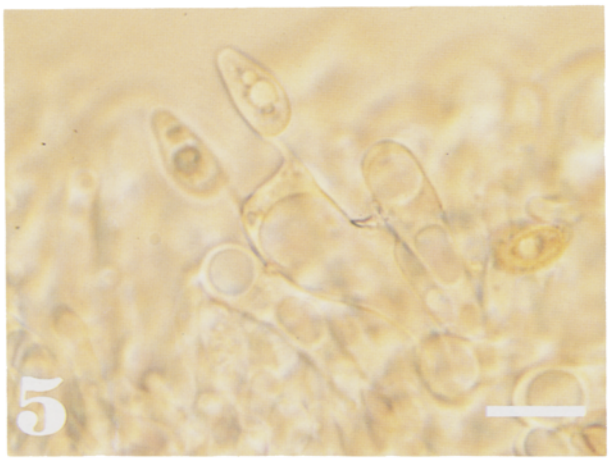
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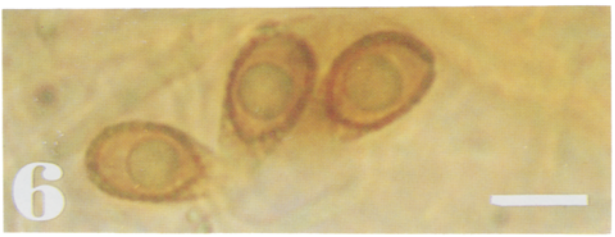
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Table 2. Effects of light and ventilation on formation of atypical fruiting structure (AFS) and fruit body primordium (FBP) of *Ganoderma lucidum*.

Treatment*	Area (mm^2) of AFS**		No. of FBP**	
	GI-009	GI-010	GI-009	GI-010
Dark Sealed	0	0	0	0
Unsealed	0	0	0	0
Light Sealed	0	0	3.3±1.2	0
Unsealed	0	3370.6±193.4	2.7±0.9	0

*Treatment is the same as that in Table 1.
** Formation of AFS and FBP was measured after incubation for 30 days. All cultures were replicated five times, and each value shows the mean with standard deviation.

skeletal hyphae and conspicuous clamp-connections on generative hyphae. The AFSs were densely organized with vegetative hyphae, skeletal hyphae and cuticular cells. Basidia differentiated directly from generative hyphae on the outside of the AFS (Fig. 9). A basidium had two to four sterigma with a hilar appendix and one or two vacuoles (Figs. 5, 9, 10). Occasionally, sterigma protruded from the side of the basidium (Figs. 5, 11).

Basidiospores were brown, ellipsoid, and had one or two large vacuoles (Fig. 5) and a double wall (Fig. 6). The surface of basidiospores was smooth or wrinkled and most had numerous small and shallow holes (Fig. 12). The surface of immature basidiospores on basidia was wrinkled only (Fig. 9). Basidiospores were truncated to narrowly rounded at the apex with an eccentric hilar appendix on a rounded spore base (Fig. 12). Length and width of basidiospores of isolate GI-005, GI-008, GI-010, GI-021, GI-026 and GI-031 were (4.5-)6.4-9.6(-10.3) × (2.6-)3.2-5.1(-6.4) μm, 7.3×4.2 μm on average. The mean spore index (ratio of spore length to width) was 1.74 (Table 4).

To compare morphology of basidiospores formed on AFSs and fruit bodies, basidiospores obtained from wild fruit bodies in Korea and Japan were observed microscopically. Their sizes were respectively 8.6-10.9×6.6-8.3 μm, averaging 10.1×7.5 μm, and 8.3-12.8×5.6-7.2 μm, averaging 10.4×6.6 μm. Their spore indexes were 1.62 and 1.58, respectively. These were larger than basidiospores from AFS, but their other morphological characteristics were almost the same (Figs. 12, 13; Table 4).

Discussion

In general, basidia and basidiospores of basidiomycetes are formed in hymenia on gills (Agaricales) or hymenial tubes (Aphyllophorales) of the fruit body. In artificial culture media, however, development of basidia and basidiospores without forming a fruit body has been reported for basidiomycetes such as *Ceratobasidium* sp. (binucleate *Rhizoctonia solani*-like fungi) (Uchida et al., 1986), *Crinipellis perniciosa* (Stahal) Singer (Bastos and Andebrhan, 1987), *Phellinus contiguus* (Pers.: Fr.) Pat. (Butler, 1988; Butler and Wood, 1988), *Ganoderma lucidum* (Shin and Seo, 1988a) and *Lyophyllum tylicolor* (Fr.: Fr.) N. Lange et Sivertsen (Yamanaka and Sagara, 1990).

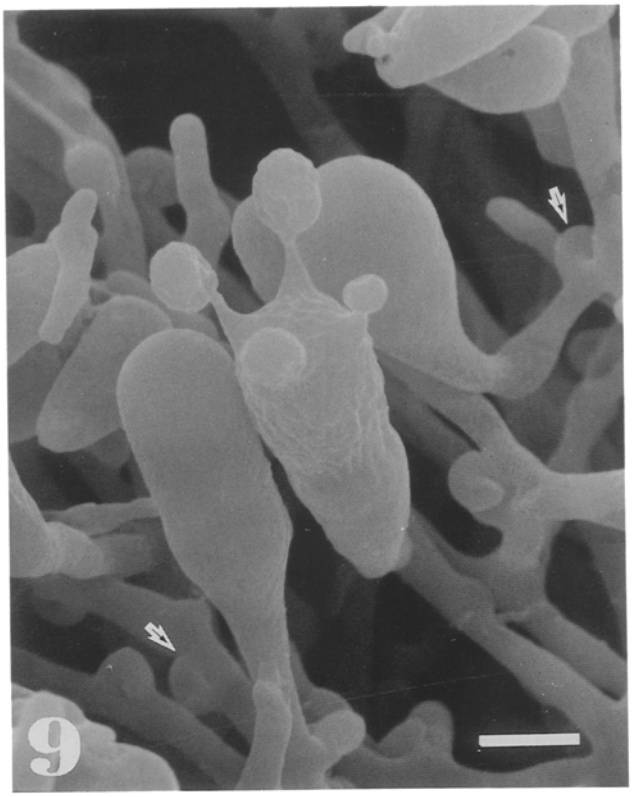
In this study, *G. lucidum* formed basidia and basidiospores on AFSs as reported previously by Shin and Seo (1988a). Both light and ventilation were necessary for formation of AFSs, which were of two types, coralloid and incomplete poroid. The type formed seemed to be affected by nutritional conditions of media and age of colony, because the same isolate formed AFSs of either

Table 3. Isolates of *Ganoderma lucidum* and their characters on culture medium.

Isolate	Source of isolate (Country)*	Character**
GI-004	Wild fruit body (Korea)	AFS
GI-005	Wild fruit body (Korea)	AFS
GI-006	Wild fruit body (Korea)	AFS
GI-007	Wild fruit body (Korea)	AFS
GI-008	Wild fruit body (Korea)	AFS
GI-010	Cultivated fruit body (Korea)	AFS
GI-019	TMI-50040 (Japan)	AFS
GI-020	TMI-50087 (Japan)	AFS
GI-021	Wild fruit body (Korea)	AFS
GI-022	ASI-7005 (Korea)	AFS
GI-023	ASI-7011 (Japan)	AFS
GI-026	Wild fruit body (Papua New Guinea)	AFS
GI-031	Wild fruit body (Korea)	AFS
GI-003	Wild fruit body (Korea)	FBP
GI-009	ASI-7018 (Japan)	FBP
GI-012	ASI-7024 (Japan)	FBP
GI-024	ASI-7016 (Korea)	FBP
GI-028	MRI 5001 (U.S.A.)	FBP
GI-002	Wild fruit body (Korea)	CLS
GI-025	Wild fruit body (Papua New Guinea)	—
GI-027	MRI 5006 (U.S.A.)	—
GI-029	MRI 5005 (U.S.A.)	—
GI-030	Cultivated fruit body (Korea)	—

* TMI: Tottori Mycological Institute, Japan; ASI: Agriculture Science Institute, Korea; MRI: Mushroom Research Institute, University of Pennsylvania, U.S.A.
** AFS: Atypical fruiting structure with basidiospores; FBP: fruit body primordium; CLS: callus-like structure without basidiospores; —; only vegetative growth.

Figs. 9-13. Scanning electron micrographs of AFS of isolate GI-010. Scales=3 μm for Fig. 9; 5 μm for Figs. 10 and 11; 2 μm for Figs. 12 and 13. 9. Four-spored basidium and clamp connections (arrowheads) on AFS. 10. Three- or four-spored basidia on AFS. Arrowhead indicates a basidium with three-sterigma. 11. Protrusion (arrowhead) of sterigma from the side of basidium. 12. Basidiospores from AFS of *G. lucidum*. Arrowhead indicates hilar appendix. 13. Basidiospores from wild-type fruit body of *G. lucidum*. Arrowhead indicates hilar appendix.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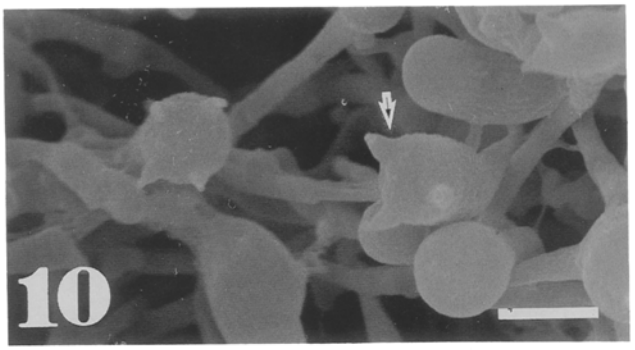
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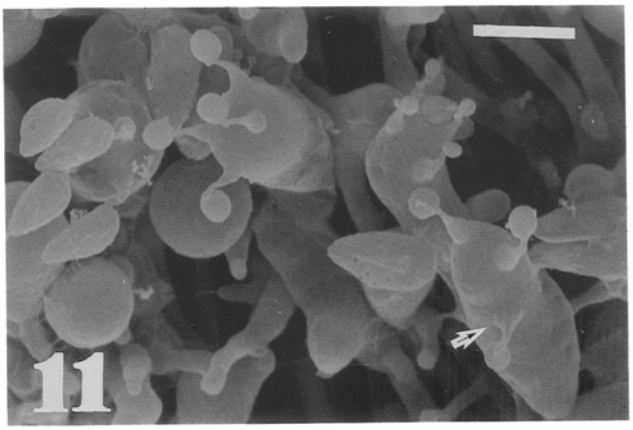
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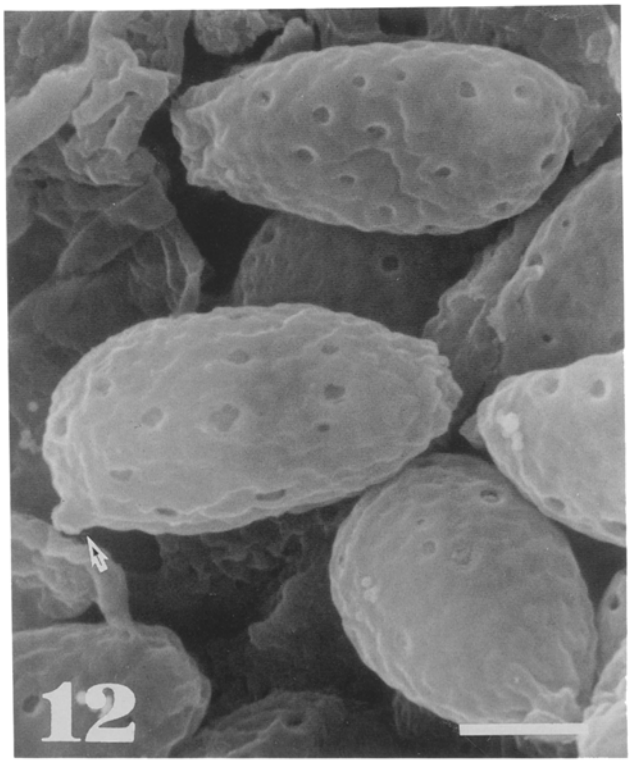
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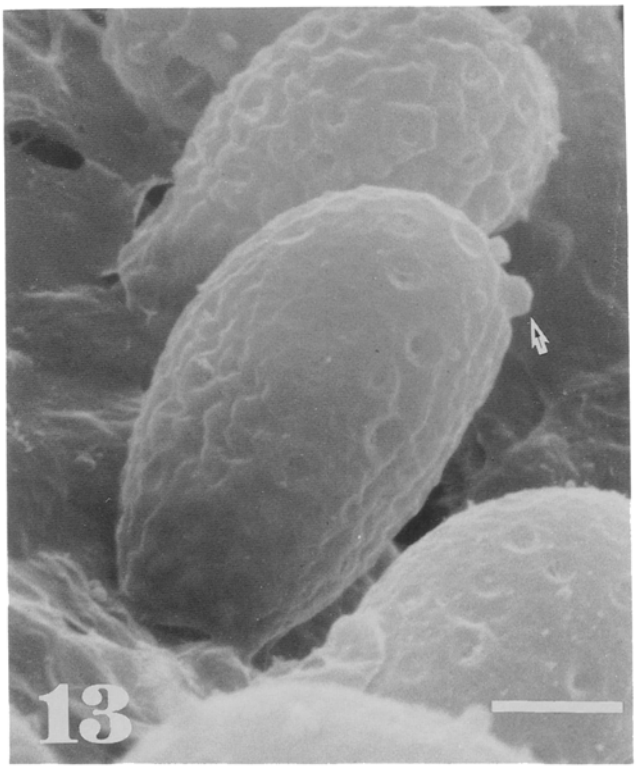
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Table 4. Morphological comparison of basidiospores formed on atypical fruiting structures and wild fruit body of *Ganoderma lucidum*.

Source	Size (μm)	Spore index*	Microscopical feature	Reference
Atypical fruiting structures	(4.5-)6.4-9.6(-10.3) × (2.6-)3.2-5.1(-6.4), 7.3 × 4.2 on average	1.74	Brown, ellipsoid with holes and eccentric hilar appendix, double wall, and vacuole	
Wild fruit body from Korea	8.6-10.9 × 6.6-8.3, 10.1 × 7.5 on average	1.62	Brown, ovoid with holes and eccentric hilar appendix, double wall, and vacuole	
Wild fruit body from Japan	8.3-12.8 × 5.6-7.2, 10.4 × 6.6 on average	1.58	Brown, ovoid with holes and eccentric hilar appendix, double wall, and vacuole	
Wild fruit body	10.6-11.8 × 6.8-7.8, 11.5-7.4 on average	1.50	Brown, ovoid with depressions and eccentric hilar appendix, double wall, and vacuole	Adaskaveg and Gilvertson (1986)
Wild fruit body	9.0-12.0 × 6.0-7.0		Ellipsoid with holes and eccentric hilar appendix	Mims and Seabury (1989)
Wild fruit body	9.5-11.0 × 5.5-7.0		Deep yellowish brown, ovoid, and double wall	Ito (1955)

* Spore index = ratio of spore length to width.

coralloid or incomplete poroid type in repeated experiments. These AFSs were organized with vegetative hyphae, skeletal hyphae and cuticular cells, and basidia were formed directly from generative hyphae on the surface of the AFS, indicating that AFSs differ clearly from fruit bodies in the formation process (Mims and Seabury, 1989). Basidiospores were brown, ellipsoid, with holes on the surface, and had a double wall, an eccentric hilar appendix on a rounded spore base, and vacuoles. The morphology of basidiospores formed on AFSs was compared with that of basidiospores obtained from natural fruit bodies in this study and from normal fruit bodies reported by Ito (1955), Adaskaveg and Gilvertson (1986), and Mims and Seabury (1989) (Table 4). Basidiospores on AFSs have the same characteristics as the normal basidiospores, except that they are smaller and slightly more slender in form. In this study, sizes of basidiospores from AFSs of six isolates and two wild fruit bodies were measured. Basidiospores from AFSs showed no difference in size among the isolates, but they were about 20 to 30% smaller than those of fruit bodies (Table 4). The reason for this difference was not clear. One possible explanation is that it resulted from the inherent characteristics of the isolates examined. Another is a difference in the developmental processes of basidiospores between AFSs and fruit bodies. Accordingly, it is necessary to compare the size of basidiospores from fruit bodies and AFSs for each isolate.

G. lucidum also formed FBPs on agar media in light. Unlike AFSs, ventilation had no effect on FBP formation. FBPs could not develop into normal fruit bodies carrying basidiospores within 30 days. Since a 3-month period of cultivation is necessary to develop fruit bodies in sawdust culture (Seo, 1987), sufficient nutritional supply in media and a long incubation period may be necessary for the FBP to develop into a mature fruit body.

It was reported that *G. lucidum* forms aberrant fruit bodies in vitro culture (Bose, 1929; Banerjee and Sarkar, 1956; Adaskaveg and Gilvertson, 1986). However, whether the aberrant fruit bodies are the FBPs observed in this study is unclear. Adaskaveg and Gilvertson (1986) reported that this fungus occasionally formed basidiospores on red laccate coral-like fruit bodies. These fruit bodies might to be AFSs, because of the similarity in their morphology and basidiospore formation.

Among 23 isolates of *G. lucidum* collected from Japan, Korea, Papua New Guinea and the U.S.A., 13 isolates (about 60% of isolates tested), none of which was from the U.S.A., formed AFSs with basidiospores, and another 5 isolates (about 20% of isolates tested), none of them from Papua New Guinea, induced FBPs. Of the remaining 5 isolates, 1 isolate from Korea formed a callus-like structure without producing basidiospores, this structure differing from AFSs and FBPs in form, and the other 4 isolates from Korea, Papua New Guinea and the U.S.A. formed neither AFSs nor FBPs. However, we found that some isolates which do not form AFSs or FBPs in single culture, formed AFSs in dual culture with *Penicillium* sp. (unpublished data). These results indicate that *G. lucidum* isolates have the ability to form either AFSs or FBPs on culture media. In artificial bottle cultivation, *G. lucidum* isolates forms either kidney-shaped or antler-shaped fruit bodies (Shin and Seo, 1988b). Isolates GI-005, GI-006, GI-007, GI-008 and GI-010, which formed AFSs in this study, formed kidney-shaped fruit bodies in bottle cultivation with sawdust. On the other hand, isolate GI-009, which formed FBPs, produced antler-type fruit bodies. The antler-shaped fruit body has a long stipe without hymenium or with an abnormal hymenium. Therefore, it may be difficult for isolates producing antler-shaped fruit bodies to form AFSs in vitro culture. Detailed studies on the formation of AFSs and FBPs in vitro culture remain to be done.

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